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Minireview

Generation and Maintenance of Neuronal Polarity: Mechanisms of Transport and Targeting

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Neurons are highly polarized cells that provide a fascinating example of how cellular morphology and accurate protein distribution lay the basis for their function: the ability to receive, process, and transmit information. Several cellular mechanisms contribute to the establishment and maintenance of neuronal polarity. As discussed in the minireview by Winckler and Mellman (1999 [this issue of *Neuron*]), protein sorting and targeting motifs, the machinery that recognizes these motifs, as well as diffusion barriers are critical, particularly in the initial phases of generating polarized cells. In this minireview, we focus on the cytoskeleton and its associated elements, motors involved in protein kinesis, and proteins important for targeting to particular subcellular domains.

Regulation of Polarized Vesicle Trafficking

Intracellular transport is essential for the generation and maintenance of neuronal polarization because it is the primary mechanism for the distribution of materials necessary for axonal and dendritic function. Many proteins are actively transported along microtubules in association with the trafficking of various membranous organelles or rafts. Studies using video-enhanced contrast light microscopy have demonstrated that organelles are transported bidirectionally in both axons and dendrites. Furthermore, electron microscopy has shown the presence of various cross-bridge structures between transport organelles and microtubules. These findings suggest the existence of an array of motor proteins that mediate the highly diverse transport systems in neurons. The identification of molecular motors such as kinesin and dynein, which are capable of movement toward the opposite ends of microtubules, provided the first glimpse of the mechanisms involved in intracellular neuronal transport. Over the last several years, multiple neuronal kinesin-related proteins involved in trafficking of distinct types of organelles have been identified (Table 1).

Within the axon, the uniform orientation of microtubules allows plus end–directed kinesins to transport cargo toward the synapse, while minus end–directed kinesins and dynein mediate transport toward the cell body. A current model proposes that minus end–directed motors are involved in transporting dendrite-specific cargo, since they will be excluded from the axon due to the orientation of axonal microtubules. The mixed microtubule polarity in the proximal portion of the dendrites would allow these motors to cycle between the cell body and dendrites. Indeed, KIFC2, the only known minus end–directed kinesin-like protein, has been shown to localize almost exclusively to the somatodendritic portion of the neurons (Saito et al., 1997). While it remains unclear which organelles are transported by KIFC2, this motor is a good candidate for dendrite-specific trafficking. However, the predominant localization of the plus end–directed motor protein (KIF21B) to the soma and dendrites appears inconsistent with this model, since such motors would be expected to eventually move into neuronal axons (Marszalek et al., 1999). Several mechanisms could account for the apparent enrichment of KIF21B in neuronal dendrites. It is possible that inactive KIF21B is transported into the dendrites by a minus end–directed motor, where it would remain sequestered until needed. Alternatively, the stability of KIF21B could be differentially regulated in axonal versus dendritic processes. Indeed, differences in protein degradation rates between axons and dendrites have been previously reported. Thus, KIF21B could enter both axons and dendrites while becoming stabilized only in dendrites, perhaps by binding to its cargo organelles.

The identification of various molecular motors involved in directional transport of specific organelles has allowed us to begin to understand the initial transport steps in the sequence of polarized trafficking in neurons. Nevertheless, many questions still remain unanswered. For example, the mechanisms regulating the dissociation of cargo organelles upon delivery to their destination remain unclear. Furthermore, and perhaps most importantly, almost nothing is known about the mechanisms by which kinesins associate with specific cargo organelles. In order to play a role in polarized trafficking, different kinesins must be able to recognize and specifically bind only the correct organelle(s). Thus, kinesins would be expected to bind to their corresponding "receptors" on the transport organelles. So far, the only such interaction identified is the binding of rab6 to rabkinesin, a novel motor protein with an as yet unidentified function (Echard et al., 1998). Thus, it will be interesting to see whether other rabs might also be involved in regulation of motor–cargo organelle interaction.

Regulation of Membrane Fusion Specificity

Upon arrival of trafficking vesicles in the vicinity of their target membranes, a set of additional mechanisms are needed to ensure their specific target recognition and docking. The syntaxin and VAMP/synaptobrevin protein families, collectively known as SNAREs, have been implicated in mediating membrane fusion through the formation of a very stable core complex. A key feature of the SNARE hypothesis is that a syntaxin interacts with an appropriate VAMP to form an organelle-specific docking complex, which ensures that transport vesicles fuse only with appropriate acceptor membranes. However, recent in vitro studies have demonstrated that syntaxin and VAMP interactions may lack the specificity needed for the vesicle–target membrane recognition. Moreover, no known syntaxin displays an axon- or dendrite-specific localization, which would be expected from a protein involved in axonal or dendritic vesicle targeting. Indeed,

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Table 1. Motor Proteins Involved in Polarized Sorting

Motor	Neuronal Localization	Direction	Transport Organelle
KIF1A[1]	axon	plus end	synaptic vesicle precursors
KIF1B[2]	ubiquitous	plus end	mitochondria
KIF2[3]	growth cones	plus end	organelles mediating axonal extension
KIF3A/B[4]	ubiquitous	plus end	unknown
KIF4[5]	growth cones	plus end	organelles mediating axonal extension
KIFC2[6]	soma and dendrites	minus end	multivesicular endosomes
KIF21A[7]	ubiquitous	plus end	unknown
KIF21B[7]	soma and dendrites	plus end	unknown

References: (1) Okada et al., 1995; (2) Nangaku et al., 1994; (3) Sekine et al., 1994; (4) Yamazaki et al., 1995; (5) Noda et al., 1995; (6) Saito et al., 1997; (7) Marszalek et al., 1999.

syntaxin 1 and SNAP-25, SNAREs that mediate synaptic vesicle exocytosis at the axonal active zones, are also present in the soma as well as dendrites of differentiated neurons. Therefore, if SNARE proteins play a role in determining the organization of membrane compartments, it might be to broadly delineate specific membrane compartments, for example plasma membrane versus endosomes. Additional regulatory proteins would then be needed to restrict the specificity of vesicle targeting and fusion within a particular membrane.

Genetic and biochemical studies in yeast have identified a protein complex that has been implicated in the targeting of secretory vesicles. This complex, known as the exocyst, consists of Sec3/5/6/8/10/15 and exo70 subunits and appears to be necessary for the efficient insertion of new membrane components to the growing tip of the yeast bud, and later to the mother–daughter cell boundary. The mammalian ortholog of the exocyst complex, often referred to as the sec6/8 complex, may play a role in the targeting of trafficking vesicles in neurons. In developing hippocampal neurons, the sec6/8 complex is enriched in growth cones and clusters on the axonal plasma membrane at periodic intervals of $\sim$3.2 μm (Hazuka et al., 1999). Early in development, these clusters colocalize with synaptic vesicle markers, suggesting that the sec6/8 complex might play a role in the targeting of synaptic proteins to the potential active zones during synaptogenesis (Hazuka et al., 1999). Interestingly, upon the formation of stable synapses, the sec6/8 immunoreactivity disappears, leaving behind a functional synapse, suggesting that the sec6/8 complex is probably not required for the local recycling of synaptic vesicles in established synapses. Perhaps the sec6/8 complex is only required when cells are undergoing active growth and targets secretory vesicles to the sites of active protein delivery and plasma membrane expansion. This could explain the indiscriminate presence of sec6/8 in growth cones of both dendritic and axonal processes. During the maturation of synapses, the developing active zones presumably receive newly synthesized proteins, in contrast to the mature synapse, which is capable of maintaining its function for some time without active biosynthetic transport from the soma.

Taken together, the data from yeast and mammalian cells suggest that the sec6/8 complex may play a role in targeting vesicles to specific membrane domains. The molecular basis for this targeting role, however, remains to be determined. In yeast, it has been suggested that the sec6/8 complex brings vesicles and plasma membrane into close proximity by simultaneously interacting with the rab GTPase Sec4p through Sec15p, and with the plasma membrane through Sec3p (Guo et al., 1999). Nevertheless, little mechanistic information is available about the sec6/8 complex in mammalian cells. It is not clear whether the mammalian sec6/8 complex contains a Sec3p analog, although the sec6/8 subunit p106 has been suggested to play that role. In addition, the sec6/8 complex is not known to interact with any of the mammalian rab proteins. Perhaps it functions in vesicle targeting through interactions with cytoskeletal proteins. Genetic studies in yeast suggest interactions between Sec3p and profilin as well as Sec6p and actin/myo2. Similarly, immunoprecipitation studies from rat brain lysates suggest interactions between the mammalian sec6/8 complex and the septins KIAA0128, CDC10, NEDD5, and H5. The septins, a novel class of cytoskeletal proteins, have been implicated in many cellular functions, including generation of cell polarity and synaptic vesicle targeting. Thus, the sec6/8 complex appears to act as a molecular scaffold bringing together cellular components necessary for efficient vesicle targeting, docking, and fusion.

The CASK/LIN-2, Velis/LIN-7, Mint/X11/LIN-10 Complex in Polarized Sorting

A growing class of proteins characterized by the presence in their sequence of one or more PDZ domains, motifs that mediate protein–protein interactions, has been shown to be involved in the organization of synaptic signaling pathways (Craven and Bredt, 1998). An emerging picture is that these proteins not only anchor neurotransmitter receptors and ion channels at synaptic sites but also provide a scaffold on which the different functional elements of the postsynaptic machinery can be assembled. For example, the protein InaD, which has five PDZ domains, provides a scaffold to tether several elements of the *Drosophila* phototransduction cascade. Similarly, PSD-95, which contains three PDZ domains, clusters and localizes NMDA-type glutamate receptors at synapses while also binding nNOS. This set of interactions is thought to link NO production and NMDA activation.

Recent studies of vulval cell differentiation in *C. elegans* demonstrated the role of PDZ domain–containing proteins in polarized protein targeting (Simske et al., 1996; Kaech et al., 1998). Three PDZ-containing proteins—LIN-2, -7, and -10—act in a concerted way to ensure the basolateral localization of LET-23 RTK, the

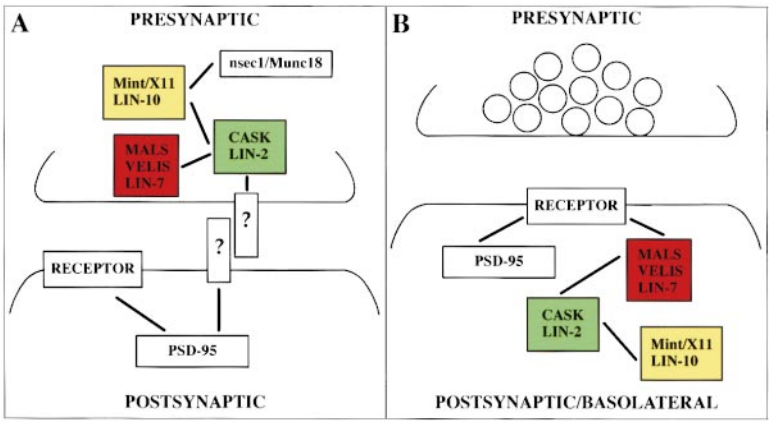

Figure 1. Potential Sites of LIN-2, -7, and -10 Function

The evolutionarily conserved CASK/LIN-2, Velis/LIN-7, Mint/X11/LIN-10 complex has been implicated in mechanisms of polarity both in presynaptic (A) and postsynaptic/basolateral domains (B) of neurons and epithelial cells. Surface proteins (?) that may mediate cell contact events during synapse formation include cadherins, integrins, and neuroligin/neurexin. See text for further discussion.

EGF receptor responsible for the initiation of the signaling cascade that leads to vulval differentiation. Mutations in any one of the genes encoding LIN-2, -7, or -10 disrupt basolateral targeting of LET-23 RTK in vulval precursor cells, thus preventing the signaling cascade and resulting in a vulvaless phenotype. Specific protein interactions between LIN-2, -7, and -10 have been elucidated: LIN-2 can bind both LIN-10 and LIN-7 to form a ternary complex. These interactions are not mediated by their PDZ domains, which remain free to bind other partners, such as the C-terminal tail of LET-23 RTK in the case of LIN-7.

Interestingly, the postsynaptic localization of the glutamate receptor GluR1 in *C. elegans* is blocked by *lin-10* mutations but not *lin-2* or *lin-7* mutations (Rongo et al., 1998), suggesting that LIN-10 is a shared component of the polarized sorting pathway in neuronal and epithelial cells. However, the functional partners of LIN-10 (whether neuronal homologs of LIN-2 and LIN-7 or other proteins) are still unknown and the mechanism for the postsynaptic targeting of GluR1 remains poorly understood.

Mammalian homologs of the LIN-2/-7/-10 complex have been identified in mammalian brain (Butz et al., 1998). CASK is the homolog of LIN-2, Velis of LIN-7, and Mint1/X11α of LIN-10. In analogy with the *C. elegans* complex, CASK binds to both Velis and Mint1/X11α independently of their PDZ domains. Interestingly, CASK was originally identified as a brain-enriched protein that can bind to the C terminus of neurexin via its PDZ domain. Similarly, Mint/X11 proteins, which form a family of three isoforms (Mint1, 2, and 3 and X11α, β, and γ) with homologous C-terminal PTB and PDZ domains but divergent N-terminal sequences, were first identified as binding partners of nsec1/Munc18.

Although the CASK/LIN-2, Velis/LIN-7, Mint/X11/LIN-10 complex is clearly evolutionarily conserved and involved in polarized protein targeting, some fundamental questions remain unanswered. Is the complex directly involved in polarized sorting and targeting of selected proteins to their final subcellular destination? Or does it play a role in tethering its interacting proteins once these have reached their appropriate location by other mechanisms? Moreover, while in *C. elegans* the complex is implicated in protein targeting to the basolateral domain in epithelial cells and to the postsynaptic specialization in neurons (LIN-10), the mammalian complex has been proposed to act presynaptically. Recent contributions have shed some light on these issues.

An antibody raised against LIN-10 was used to investigate its subcellular distribution in late L3 larvae of *C. elegans* (Whitfield et al., 1999). By comparing the staining of LIN-10 with that of a marker for the *trans* cisterna of the Golgi complex, the authors concluded that, in addition to its presence at the plasma membrane and in the cytoplasm, a major pool of LIN-10 is localized in the *trans* cisterna of the Golgi complex or in the *trans*-Golgi network. Antibodies against the homologous mammalian protein Mint1/X11α recognize intracellular compartments and stain processes in a pattern suggestive of synaptic localization (Borg et al., 1999). Moreover, when differentiated NT2 cells are immunostained with anti-Mint1/X11α antibodies, the protein is found in the cytosol and in the perinuclear region, as is CASK. Considerable colocalization of Mint1/X11α with giantin, a Golgi complex marker, suggests that there is a pool of Mint1/X11α and CASK localized in the Golgi apparatus. Thus, the subcellular localization of LIN-10 and Mint1/X11α is consistent with both a role in tethering the interacting protein(s) at the target membrane and a role in sorting membrane proteins, at the level of the Golgi complex, into distinct trafficking vesicles destined for different target membranes.

While the presynaptic role of the mammalian complex is supported by the localization of some of the known binding partners for CASK and Mint1/X11α—neurexin and nsec1/Munc-18, respectively—several lines of evidence suggest that the mammalian complex might have additional or alternative postsynaptic roles (Figure 1). First, CASK has been shown by both light and electron microscopy to be distributed in a punctate somatodendritic pattern in neurons. The protein is enriched, but not exclusively localized, at synapses and is also present at nonsynaptic plasma membranes and on intracellular compartments. At the synapse, CASK is found in both presynaptic and postsynaptic compartments with a moderate enrichment in postsynaptic density fractions (Hsueh et al., 1998). Second, as described above, Mint1/X11α is distributed in a punctate somatodendritic pattern in neurons. Third, Mint1/X11α can restore the postsynaptic localization of GluR1 in *lin-10* mutants (Rongo et al., 1998), suggesting, by analogy, a role in postsynaptic targeting or tethering of vertebrate glutamate receptors. Recently three proteins, MALS-1, -2, and -3 (mammalian LIN-7 proteins), which are identical to the Velis,

have been characterized (Jo et al., 1999). MALS-1 and -2, the neuron-specific isoforms, were shown by biochemical fractionation to be highly enriched in postsynaptic density preparations. Immunofluorescent labeling of cultured hippocampal neurons revealed a punctate somatodendritic pattern suggestive of a synaptic localization, which was confirmed by double labeling with antibodies against PSD-95 and an NMDA receptor subunit (NR1), two markers of the postsynaptic density. Furthermore, both immunoprecipitation experiments and pull-down assays indicated that PSD-95 and NR2B, another subunit of the NMDA receptor, selectively interact with MALS, the latter being a classic PDZ-mediated interaction. These data provide strong evidence for a postsynaptic role of the CASK/Velis/Mint1/X11α complex.

In conclusion, the past few months have furthered our understanding of the function of the CASK/LIN-2, Velis/LIN-7, Mint/X11/LIN-10 complex. These proteins comprise an evolutionarily conserved complex involved in fundamental aspects of protein targeting in neurons and epithelia. The structural homology is now shown to be accompanied by functional homology: as shown for LIN-7 and LET-23 RTK, the mammalian Velis/MALS also directly bind via a PDZ interaction to a plasma membrane protein, the NR2B subunit of the NMDA receptor. While these interactions suggest a role for Velis/MALS in postsynaptic targeting of neuronal receptors, direct proof is still lacking and knockout and dominant-negative approaches aimed at further delineating the function of Velis/MALS are needed.

Several interesting questions remain. Analysis of the subcellular localization of the components of the complex revealed their presence at both the specific target, the plasma membrane, and in the Golgi complex region, possibly the *trans*-Golgi network. Thus, maybe the complex first participates in the sorting process of selected plasma membrane proteins into distinct transport vesicles at the level of the *trans*-Golgi network and then plays a later role in tethering vesicles or proteins at the target membrane. Perhaps the components of the complex are not always fully assembled and, aside from their role as a complex, have individual functions at different steps during the targeting process. In addition, it is likely that assembly of the complex is highly regulated, and perhaps fully functional states are only achieved at the target membrane, similar to the sec6/8 complex.

In the *lin-2*, *-7*, and *-10* mutants in *C. elegans*, neither the vulval epithelial cells nor the neurons are grossly affected in their general polarity. The vulval precursor cells maintain distinct apical and basolateral membrane compartments that correctly secrete components of the cuticle (apically) and components of the extracellular matrix (basolaterally). Similarly, neurons in *lin-10* mutants respond to stimuli that require activation of receptors distinct from GluR1. These results suggest that multiple mechanisms function to ensure polarized sorting of cellular components. What are these other mechanisms? How do they interact with the LIN-2/-7/-10 complex, and does this complex interact with the sec6/8 complex?

As further molecules important for cell polarization are discovered and their mechanisms of action become understood, it is likely that a multifaceted regulatory network, beginning with sorting processes in the secretory pathway and ending at the target membrane of the differentiated cell, will be unveiled.

Selected Reading

Borg, J.-P., Lopez-Figueroa, M.O., de Taddeo-Borg, M., Kroon, D.E., Turner, R.S., Watson, S.J., and Margolis, B. (1999). J. Neurosci. *19*, 1307–1316.

Butz, S., Okamoto, M., and Südhof, T.C. (1998). Cell *94*, 773–782.

Craven, S.E, and Bredt, D.S. (1998). Cell *93*, 495–498.

Echard, A., Jollivet, F., Martinez, O., Lacapere, J., Rousselet, A., Janoueix-Lerosey, I., and Goud, B. (1998). Science *279*, 580–585.

Guo, W., Roth, D., Walch-Sollimena, C., and Novick, P. (1999). EMBO J. *18*, 1071–1080.

Hazuka, C.D., Foletti, D.L., Hsu, S.-C., Kee, Y., Hopf, F.W., and Scheller, R.H. (1999). J. Neurosci. *19*, 1324–1334.

Hsueh, Y.-P., Yang, F.-C., Kharanzia, V., Naisbitt, S., Cohen, A.R., Weinberg, R.J., and Sheng, M. (1998). J. Cell Biol. *142*, 139–151.

Jo, K., Derin, R., Li, M., and Bredt, D.S. (1999). J. Neurosci. *19*, 4189–4199.

Kaech, S.M., Whitfield, C.W., and Kim, S.K. (1998). Cell *94*, 761–771.

Marszalek, J.R., Weiner, J.A., Farlow, S.J., Chun, J., and Goldstein, S.B. (1999). J. Cell Biol. *145*, 469–479.

Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamazaki, H., and Hirokawa, N. (1994). Cell *79*, 1209–1220.

Noda, Y., Sato, Y.R., Kondo, S., Nangaku, M., and Hirokawa, N. (1995). J. Cell Biol. *129*, 157–167.

Okada, Y., Yamazaki, H., Sekine-Aizawa, Y., and Hirokawa, N. (1995). Cell *81*, 769–780.

Rongo, C., Whitfield, C.W., Rodal, A., Kim, S.K., and Kaplan, J.M. (1998). Cell *94*, 751–759.

Saito, N., Okada, Y., Noda, Y., Kinoshita, Y., Kondo, S., and Hirokawa, N. (1997). Neuron *18*, 425–438.

Sekine, Y., Okada, Y., Kondo, S., Aizawa, H., Takemura, R., and Hirokawa, N. (1994). J. Cell Biol. *127*, 187–202.

Simske, J.S., Kaech, S.M., Harp, S.A., and Kim, S.K. (1996). Cell *85*, 195–204.

Whitfield, C.W., Benard, C., Barnes, T., Hekimi, S., and Kim, S.K. (1999). Mol. Biol. Cell *10*, 2087–2100.

Winckler, B., and Mellman, I. (1999). Neuron *23*, this issue, 637–640.

Yamazaki, H., Nakata, T., Okada, Y., and Hirokawa, N. (1995). J. Cell Biol. *130*, 1387–1399.